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The Influence of Acridine Compounds on the Preparation of Smallpox Vaccine Virus, by N. A. Zeytlenok, E. P. Pille and O. V. Konosh

Since 1945 there have been published numerous studies[1] in which acridine was used for the study of the physiology of reproduction of some viruses. Analysis of literature indicates that one and the same acridines diversely affect the viruses present and in the reverse, one and the same viruses behave differently in the presence of acridines. The data at hand refer to the odd character and sometimes to the discrepancies. In a majority of the cases the authors limit their statements to whether or not one or another acridine suppresses the virus being studied. The mechanism of action of the acridines on the multiplication of the virus is poorly studied. It has been established that rivanol acts overwhelmingly on virus of tobacco mosaic and phages, but there is practically nothing of the action of this preparation on the viruses of man and animals. Only two works are devoted to the effect of acridines on virus of smallpox, according to which the atebrin in the cultures of surviving tissues and proflavine in chicken embryos overwhelms the virus (2, 3).

This work contains two problems: Study the action of several acridine preparations on virus of smallpox and clarify several conditions under which the acridines render a suppressing action on the multiplication of that virus.

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1. Rezhkov, V. I.; Smirnova, V. A.; Goroshkaya, S. S. Book: Refer. work of the Dept. of Biol. Sci., Acad. Sci. USSR, 1944. M., 1945. Pages 73-74.
2. Thomas, R. L. J. Immunol. V.55, No. 4, p.345-352, 1947.
3. Briody, B. A. and Stannard, C. J. Immunol. V.67, No. 5, p.423-430, 1951.

The virus of smallpox was cultivated on chorioallantois membranes of chicken eggs which were 10-12 days old. In the first tests the presence of a specific infection was determined on the basis of detection of visible areas of affection on the membrane; in the preparations from the membranes - characteristic intracellular impurities and Hayems' (elemental) corpuscles; positive reactions of hemoagglutination with extracts of the membranes; development of an intracutaneous reaction in rabbits and, development of keratitis in rabbits and guinea pigs with formations of intracellular impurities and elemental (Hayems) corpuscles.

But, because during the utilization of all the above methods identical results were obtained, in further tests we computed the tests mainly on the visible areas of affection on the chorioallantois membrane, and results of the hemoagglutination reaction.

The acridine preparations were diluted with a phosphate buffer (pH 7.2) or in a physiological solution of sodium chloride or with distilled water. 0.1 ml of the solution was applied to the chorioallantois membrane and 5 to 15 minutes later 0.1 ml of the suspension of the virus was applied to the same place on the membrane. A corresponding solution was applied to the control embryos in place of the acridines. The infected embryos were incubated for 48 hours at 35.5° after which they were opened. Visible areas of affection were noted on the membranes and then suspensions of the membrane were prepared in physiological solutions. After centrifugation in a supercharged fluid, a determination of the hemoagglutinins to the chicken erythrocytes was made. In all tests the results for the determination of virus through the presence of visible affections and by the reaction of the hemoagglutination quite significantly coincided; therefore, in order to economize, only the data of this

reaction is considered.

The tests were conducted with nine acridine preparations. Rivanol, trofiavin and etebrin were tested more than the remainders. The results of these tests are summarized in Table 1 (end of text). The obtained data, statistically reliable, give evidence of the suppressing action of the rivanol, trofiavin and etebrin on the virus of smallpox. It was also established that other acridines, especially acridine yellow, acriphosphine and 7-nitroacrichine (7-nitroetebrin), are able to suppress the development of the virus.

In numerous tests it was clear that certain conditions effect the suppressing action of rivanol.

The first item to be studied was the importance of the path of injection of the preparation into the embryo. As Table 2 indicates, during injections of the rivanol into the amniotic cavity and yolk sac the suppressing action on the development of the virus in the chorioallantois membrane of the chicken embryos was not observed; it was observed only when the preparation was applied to the chorioallantois membrane or injected into the allantois cavity. This is reasonable basis to think that the desired suppressive action can be obtained by creating a sufficiently high concentration of large doses of rivanol directly in those tissues in which the multiplication of the virus is taking place.

Determination was also made on the action of various size doses of rivanol applied on the chorioallantois membrane 5-15 minutes before the introduction of the virus. Data on Table 3 show that the 0,5 mg dose rendered a distinct suppressive action on the virus. A half dose acted somewhat weaker. Decreasing the dose to 1/4 and 1/8 gave indicators which were barely distinguishable from

the indicators of the control group of embryos.

The question arose as to whether or not the suppressive action of the rivanol and other acridines does not amount to a simple inactivation of the virus at the place of introduction. Several tests were conducted to study the action of rivanol and other acridines on the virus of smallpox in vitro.

Equal quantities of virus in dilutions 10^{-2} and 0,5% solution of rivanol or 2% acrichine in a phosphate buffer at pH 7,2, were mixed together and held for 18 hours at 4°C or 2 hours at 36°C. Then we prepared from these mixtures tenfold dilutions and applied 0,2 ml to the chorioallantois membrane of the embryos.

As data of Table 4 indicate, the activity of the virus in the higher dilutions (where too small a quantity of acridines reached the embryo) decreased very little in comparison with the activity of the controls. In reverse, the lower dilutions of virus (10-2) rendered a distinct suppression on the virus, in comparison with the controls. These data surprisingly indicate that rivanol and acridine do not inactivate the virus of smallpox during contact in vitro.

The influence of rivanol on the development of the infection activity of the virus was also clarified.

Ten embryos were infected with virus. Five of the embryos received 0,5 ml of rivanol before the infection, the other five - a phosphate buffer solution. After 48 hours of incubation in a thermostat the chorioallantois membrane from each group of embryos were mixed, prepared into suspensions and titrated by the usual means, in twofold dilutions in a reaction of hemagglutination, and in tenfold dilutions for injection into chicken embryos.

Comparative data of the determination of the titer of reaction of hemaggln-

titration and infection titer for the chicken embryo (Table 5) indicate that during a full suppression of the hemagglutination titer there is a simultaneous stopping of the development of the infection titer of the virus. A decrease of the infection titer the virus is fully authentic. Consequently, the action of the virucol does not lead to the direct inactivation of the virus, nor to the destruction of the cells of the chorioallantoic membrane in which the virus is developing. Apparently the tissue of the membrane of the embryo is not subjected to the influence of virucol, but a condition creates itself within the tissue which prevents the development of the virus.

In further tests, the dependence of the action of the virucol on the period of the introduction was studied. In a series of tests the virucol in doses of 0.5 mg was applied to the chorioallantoic membrane 12 hours before the introduction of the virus, simultaneously with the virus and 3, 5, 7, 8, 10 and 24 hours after the introduction of the virus. A buffer solution was used instead of the virucol for a control in these same periods. It became evident that the virucol possesses a distinct suppressive action not only during a simultaneous introduction of it and the virus. A significant suppression of the virus was observed during prophylactic introduction of the virucol 12 hours before the virus and also during 'medical' application in periods up to 10 hours after infection. This material once again confirms that virucol renders the virus of smallpox a certain 'virostatic' action rather than a 'virucidal'. The possibility of obtaining a suppressive effect from the application before and after the action serves as basis for tests of utilization of this mechanism in chemotherapy and chemoprophylaxis of virus of infections.

CONCLUSIONS

1. In repeated tests statistically authentic data were obtained concerning the ability of rivanol, proflavin and acrichine to fully suppress the development of the hemagglutinins of virus of smallpox and also the development of visible affections in the chorioallantois membrane of chicken embryo. The suppressive action of acridine yellow, euflavine and 7-nitroetebrin was also established. ( )

2. During injection of rivanol into the amniotic cavity and yolk sac there was not observed any suppressive action on the development of the virus of smallpox in the chorioallantois membrane of chicken embryo. It appears only during application of the preparation directly on the chorioallantois membrane or upon introduction into the allantoic cavity. Evidently a sufficiently high concentration of acridine at the place of multiplication of the virus is necessary for the attainment of a desired suppressive action.

3. Holding a mixture of virus of smallpox together with a solution of rivanol, or acriabine, for 18 hours at 0°C, or for 2 hours at 36°C, followed by a preparation of dilutions of this mixture and injection into chicken embryos we established that a contact with the above preparations in vitro does not influence the infection titer of the virus.

4. During application of rivanol on the chorioallantois membrane there is observed a significant weakening of the infectious properties of the virus of smallpox (decrease of the titr to 219 times) besides the full suppression of the development of hemagglutinins.

5. Rivanol renders a distinct suppressive action on virus of smallpox when applied on the chorioallantois membrane from 18 hours before infection up to 18 hours after.

6. The inability of rivanol and novichine to inactivate virus during contact in vitro, the presence of a distinctive suppressive action in vivo during introduction of the preparation in comparatively remote periods before and after infection of the embryo by the virus and the possibility of a partial multiplication of the virus in the presence of the said preparations indicates that the suppressing action of rivanol and novichine on the multiplication of virus of smallpox in the chorioallantoic membrane of chicken embryos is by character virostatic and not virucidal.

Notation to Table 1: Denominator - number of infected embryos; Numerator - number of embryos with a positive reaction of hemagglutination. The statistical authentiey of difference of the indicators determined according to A. I. Boyarskii; Tables for determination of authentiey of statistical indicators and number of observations in a statistical study. M., 1947.

Table 1. Influence of rivanol, proflavin and acrichine on the development of virus.

Preparation.	Test: Result of infection.	Test: %	Control: Result of infection.	Control: %	Diff. between control & test.	Minimum statistic authentic differ.
Rivanol 5.0 (10 tests)	4/113	4	72/108	67	63	7.5
Proflavin (7 tests)	16/54	30	38/51	75	45	19
Acrichine 2.0 (8 tests)	9/56	14	45/69	70	56	14

Table 2. Action of rivanol during various paths of introduction into the embryo.

Path of introduction	Dose in mg	Number of infected embryo	Number with positive reaction of hemagglutination
Chorioallantois membrane	0.5	10	2
Allantois cavity	0.5	10	1
Amniotic cavity	0.5	10	9
Yolk sac	1.0	19	19
Control	—	19	16

Table 3. Influence of various doses of rivanol on virus development.

Dose in mg	Number of embryo.	Num with positive rea[1]: Number	Num with positive rea[1]: %	Diff. cont. & test.	Minimum statis/ authen. diff.
0.5	18	1	6	66	24
0.25	17	3	17	55	28
0.125	18	11	61	11	27
0.0625	18	11	61	11	27
Control	18	13	72	—	—

1. Reaction of hemagglutination

Table 4. [illegible] of action of rivanol and acrichine on virus in vitro.

Contact conditions	Preparation	Dose in mg % to result	Dilution of virus (logarithm) Test 2.0	3.0	4.0	5.0	Control 2.0	3.0	4.0	5.0
18 hrs 4 C.	Rivanol	Dose	0.5	0.05	0.005		---	---	---	---
		Results	1/6	3/7	3/6		5/7	2/5	1/6	---
2 hrs 36 C.	Rivanol		0.5	0.05	0.005	0.0005	---	---	---	---
			1/10	5/10	6/10	3/10	9/10	7/9	8/10	2/9
2 hrs 36 C.	Acrichine		2.0	0.2	0.02	0.002	---	---	---	---
			1/6	6/7	7/8	5/7	7/8	6/8	8/8	3/6

Footnote.. Domoninator--number of infected embryos: Numerator--number of embryo with positive reaction of hemagglutination

Table 5. Influence of rivanol on development of hemagglutinational and infectional titer of smallpox virus.

Treatment of embryo	Titer MM	Titration of infectionous (logarithm dilution) 1.0	2.0	3.0	4.0	5.0	6.0	7.0	Lg LD50	Infection titer	Indicator of suppression
Rivanol 0.5 mg	0	3/4	3/4	2/4	1/4	1/3	0/4	0/4	3.0	1 000	219
Phosphate buffer	1220	4/4	4/4	3/4	3/3	3/4	1/4	0/4	5.34	219 000	----

Explaination same as Table 4.